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Origin and Correction of Magnetic Field Inhomogeneity at the Interface in Biphasic NMR Samples

Bryan T. Martin, G. C. Chingas, and Owen M. McDougal

Department of Chemistry and Biochemistry, Boise State University, Boise, Idaho 83725

Bryan T. Martin: bryanmartin@u.boisestate.edu; G. C. Chingas: gerrychingas@boisestate.edu; Owen M. McDougal: owenmcdougal@boisestate.edu

Abstract

The use of susceptibility matching to minimize spectral distortion of biphasic samples layered in a standard 5 mm NMR tube is described. The approach uses magic angle spinning (MAS) to first extract chemical shift differences by suppressing bulk magnetization. Then, using biphasic coaxial samples, magnetic susceptibilities are matched by titration with a paramagnetic salt. The matched phases are then layered in a standard NMR tube where they can be shimmed and examined. Line widths of two distinct spectral lines, selected to characterize homogeneity in each phase, are simultaneously optimized. Two-dimensional distortion-free, slice-resolved spectra of an octanol/water system illustrate the method. These data are obtained using a 2D stepped-gradient pulse sequence devised for this application. Advantages of this sequence over slice-selective methods are that acquisition efficiency is increased and processing requires only conventional software.

Keywords

NMR; MAS; magic angle spinning; susceptibility; biphasic; shimming; demagnetization field; octanol; bulk magnetism; chemical shift

1. Introduction

Biphasic phenomenon have been studied to understand reaction kinetics [1], equilibria [2], partition coefficients [3], surfactant aggregation [4], prediction of reaction mechanism [5], and investigation of biological membrane models [6, 7]. Techniques other than NMR used to investigate surface properties of biphasic systems include atomic force microscopy [8], scanning electron microscopy [9], nonlinear optics [10], scanning electrochemical microscopy (SECM) [11], and x-ray [12] and neutron [13] scattering. A number of methods have been proposed for generating slice-resolved spectra of biphasic systems prepared in conventional NMR tubes, and run in high resolution liquid probes equipped with z-gradients [3, 14–16]. Here we study consequences of, and remedies for, magnetic field inhomogeneity caused by susceptibility mismatch at the interface.

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Correspondence to: Owen M. McDougal, owenmcdougal@boisestate.edu.

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Placing immiscible phases in an NMR tube to obtain spectra from axial slices at first appears to be an elegantly simple way to study *in situ* spatial properties of reactions and transport. However, a number of complexities are revealed by closer examination.

Kozminsky's first demonstration of frequency-selective slice spectroscopy recognized the inherent conflation of spatial and spectroscopic variables from the outset in his pioneering work [14]. Mantel et al. dealt with this latter issue by offering a program which shears the data sets to cleanly separate spectral and spatial coordinates. They further accounted for axial probe sensitivity variation by weighting intensities of slices according to the experimentally determined probe profile [16]. Lambert et al. [15] showed that 1 μm slice resolution is feasible. They employed slices which were transversely restricted to the central flattest part of the meniscus, additionally improving registration between the curved interface and flat selected slice.

Other issues remain. One is whether slice volumes are large enough to provide adequate signals from lower-concentration-surface-active species [17]. A second is that it can be difficult to isolate diffusive transport from unanticipated slow convection [16, 18], and slice-selection studies [3] have yet to take this into account. A third problem, which we address here, is line shape distortion in the vicinity of the interface caused by susceptibility mismatch between phases. Our approach is to use paramagnetic doping to make the interface between the aqueous and the organic phases magnetically invisible. Matching the magnetic susceptibility of the bulk phases by doping, prior to slice-selective studies, provides clean NMR slice spectra up to and at the interface. Additionally, we present data using a 2D pulse sequence based on stepped-gradients. This approach entirely avoids shearing of the chemical shift/position correlation common to frequency-selective methods [14, 16], while offering sensitivity advantages analogous to those of Fourier over slow passage NMR spectroscopy [19].

1.1. Theory

Consider a biphasic system composed of organic and aqueous solvents layered in an NMR tube. The NMR frequency of an analyte present in both phases, such as acetone used in the present example, will vary spatially from one phase to the other. The local frequency is determined by two components. The first is the chemical shift (CS) associated with perturbation of electronic wave functions due to differences in the chemical environment unique to each solvent [20]. This shift is uniform within each phase, and changes abruptly from one side of the interface to the other.

The second is the bulk magnetic shift (BMS) of the internal magnetic induction $\vec{B}$ that depends on the solvent's magnetic volume susceptibility κ ,¹ the symbol κ is used throughout to denote volume susceptibility, in contrast to molar susceptibility commonly denoted by χ . Unfortunately, in electromagnetism texts, χ denotes bulk susceptibility[22]; we will avoid this latter usage. The discontinuity in κ in biphasic systems distorts both field and line shape near the interface.

To a first degree of approximation, the effect of BMS susceptibility is as follows. The NMR magnet wire currents generate a magnetic field intensity H_0 throughout the sample volume. H_0 is homogeneous at the ppm level, and induces a correspondingly uniform sample magnetization $M = \kappa H_0$. Therefore, the internal magnetic induction B , which determines the NMR frequency, is shifted from the free-space value $\mu_0 H_0$ (in SI units) to

¹Following the NMR susceptibility literature [21].

$$\vec{B} = \mu_0 (\vec{H}_0 + \vec{M}) = \mu_0 (1 + \kappa) \vec{H}_0 \quad (1)$$

For homogeneous samples, the Z_0 field compensation can bring the sample resonance to the magnet's specified γB frequency regardless of κ . However, for biphasic samples, since different susceptibilities are present in each sample section, analogous field compensation would require an H_0 profile with an abrupt step at the interface.

Second degree corrections, due to a “demagnetization field” [22], modify this picture slightly. This additional field arises from an effective magnetic charge density $\hat{n} \cdot \vec{M}$ residing on material surfaces [23, 24]. High resolution NMR samples situated coaxially in the magnet therefore appear to have magnetic surface charge densities $\pm \kappa H_0$ at top and bottom. Additionally, molecules in liquids do not experience the entire average field, to which they contribute, but the field inside a Lorenz sphere [25] which effectively excludes the field of the molecule itself. The net effect is reduction in the NMR susceptibility shift equivalent to dividing κ by 3.

For homogeneous NMR samples, whose ends lie outside the detection region, the effect of end charges is correctable by shimming. A residual uniform field shift is accounted for by the shape factor α discussed in susceptibility studies [26, 27]. For biphasic samples, however, the susceptibility discontinuity at the interface effectively places an extra magnetic charge monolayer $\Delta \kappa H_0$ directly in the middle of the detection window. A plot of the resulting magnetic field, modeled using Maple 5.1, is shown in Fig. 1. Note that the demagnetization field from the charge layer is partly beneficial. The field step predicted by the first order discussion above is softened to a gradual transition that occurs over an axial length on the order of the radius of the NMR tube. However, the axial field gradient steepens with increasing distance from the tube axis, so a radial gradient is also present. Therefore, attempts to circumvent interfacial line broadening due to the axial gradient by decreasing slice thickness will fail, unless slice extent is reduced transversely as well.²

For the n-octanol/water system discussed below, we determined the interphase field step due to the BMS alone to be -169 Hz. In a standard NMR tube with 2.5 mm radius, the axial field gradient at the interface will be $169/2.5 \approx 70$ Hz/mm. *Here lies the problem:* spectra from slices near the interface will be significantly broadened, and the strength and short range of the gradient make shim correction impossible.

1.2. Approach

To eliminate line shape distortion caused by the presence of the interfacial gradient, we propose removing the susceptibility difference between the two solvents entirely. For comparison, we have converted molar susceptibilities of organic compounds listed in the CRC [28], for comparison with H_2O . For convenience, we also reproduce some data provided by other workers ([21] J. W. Emsley, J. Feeney and L. H. Sutcliffe, *High Resolution Nuclear Magnetic Resonance Spectroscopy* (Pergamon, Oxford, 1965)). Inspection of this data shows that the -9.04 and -8.86 PPM diamagnetic SI susceptibilities of water and D_2O , respectively, are larger than that of many organic solvents.³ This suggests the strategy of paramagnetically doping the aqueous phase. Paramagnetic adjustment is common in high-resolution probe design [29, 30], and more general matching methods are in use for balancing solvent silica susceptibilities in NMR chromatography [31]

²Notably, Lambert et al.'s thin restricted voxel satisfies both these conditions.

³Notable exceptions are halogenated and aromatic compounds. In these cases, matching by diluting the organic phase with a very weakly diamagnetic solvent is appropriate using similar laboratory procedures.

We illustrate this technique for an n-octanol/D₂O biphasic system, using GdCl₃ as the dopant. The system was chosen not only because it is a classic system for pharmaceutical partition coefficient studies [32], but also because it is an example of a system with susceptibilities that are difficult to predict from first principles. Although paramagnetic dosage might be calculable for sufficiently well-characterized systems, thus avoiding the need for the HRMAS measurements below, susceptibilities of the phases are not always known. In the case here, the large excess molar volumes of alcohol/water mixtures [33, 34], and the presence of D₂O in place of water, make prediction of the volume susceptibility of the octanol phase difficult.

Because water and n-octanol are partially miscible, in equilibrium, GdCl₃ is present in both phases, and both phases must be doped together. We therefore add 0.1% V/V acetone in all samples as a field reference, and define the shift difference $\Delta f = f_A - f_O$, where f_A and f_O are acetone frequencies in aqueous and organic phases, measured in the same H_0 field. At each doping level, we resolve Δf into chemical and magnetic differences according to $\Delta f = \Delta f_{CS} + \Delta f_{BMS}$. The differences Δf_{CS} depends on chemical environment and is fixed for low dopant concentrations, while Δf_{BMS} depends on doping. Susceptibility is matched when $\Delta f_{BMS} = 0$.

Figure 2 presents our methodology. First, magic angle spinning (MAS) which removes BMS is used to measure Δf_{CS} directly in undoped organic and D₂O phases. Second, Δf is measured over a range of dopant concentrations, with phases coaxially separated by a capillary, which subjects both phases to the same H_0 field. When doping makes Δf the same as the MAS Δf_{CS} , the BMS disparity is negated and susceptibilities are matched. Third, the phases are doped to this level and placed in the layered configuration for slice-resolved spectroscopic characterization. Analytes of interest may be added to the appropriate phase(s) just prior to this final stage, taking care to leave the susceptibility negligibly perturbed.

2. Experimental

2.1. Determining CS Difference By MAS

MAS is widely used to suppress line broadening due to internuclear dipolar coupling [35]. Because BMS also arises from dipolar coupling, albeit electron-nuclear, MAS experiments also suppress BMS [21, 24, 36–39]. We discuss here how this allowed us to determine that Δf_{CS} , the acetone shift difference between phases, was +67 Hz. The methyl protons are thus more shielded in the organic phase than in the aqueous.

The two phases could in principle be run at once in a single MAS rotor, but here each phase was run sequentially, to clarify assignment of the acetone resonance in D₂O and octanol phases. Accordingly, two separate 50 μ L capacity 4 mm O.D. rotors, one with D₂O, and the other with D₂O-saturated octanol. The D₂O rotor was placed in a Bruker K3168 MAS probe, and the system shimmed, tuned and spun at 3 kHz. With lock off, Z_0 was manually adjusted for the first sample to bring the water resonance to 4.7 ppm. A spectrum was recorded, and the D₂O rotor was ejected and replaced with the octanol-filled rotor. The MAS spectrum of the octanol was then recorded, without retuning or moving probe components that might have inadvertently altered the sample field. Measurements were alternated to obtain three acetone spectra each in D₂O and octanol phases for subtraction. The average frequency difference between phases was the 67 Hz quoted above, with about 2 Hz random scatter over the 30 min measurement period. (Measured magnet drift rate is 4 Hz/day.)

2.2. Doping Adjustment

NMR doping studies were performed using combinations of three stock solutions: D₂O, n-octanol, and 250 mM GdCl₃ dissolved in D₂O. All reagents were obtained from Sigma Aldrich (~99% purity). Octanol stock solution was prepared by combining D₂O with n-octanol in a 3:7 ratio, vortexing for one min., followed by overnight equilibration to ensure separation of layers. The D₂O-saturated octanol layer was then extracted and used as the octanol stock for the organic phase. The 250 mM GdCl₃-doped stock was prepared by dissolving 0.465 g of GdCl₃ · 6 H₂O into 5 mL of D₂O. These stock solutions were used for all subsequent experiments.

The miscibility of GdCl₃ in both phases required careful preparation to achieve reproducible doping levels. Here, a series of samples were prepared from the 250 mM GdCl₃ stock, by dilution with D₂O to provide starting GdCl₃ concentrations of 0, 1, 5 and 10 mM. An equal volume of octanol stock was then added to each. The samples were vortexed for 15 sec., and allowed to phase-separate for 30 min. Shift differences of the acetone lines in the two phases in each sample were then compared.⁴

After equilibration, the phases were placed in coaxial tubes (Wilma Labglass #519), consisting of a 1.52 mm I.D. capillary tube centered inside a 5 mm NMR tube (Fig. 2B). From the equilibrated aliquots, 80 μ L of the octanol phase was transferred to the capillary and 400 μ L of the aqueous phase to the outer NMR tube region.

The coaxial tube samples for each starting concentration of GdCl₃ were locked on D₂O, and shimmed using 1D Topshim. The acetone shift difference from the resulting ¹H NMR spectrum was then measured. Figure 2A shows the spectrum obtained for [GdCl₃] = 0 mM. The stronger acetone peak at 2.20 ppm is assigned to the larger-volume aqueous phase. In this undoped sample, the aqueous acetone line is 102 Hz upfield of its octanol counterpart, so $\Delta f = 102$ Hz. This implies that the undoped Δf_{BMS} is $\Delta f - \Delta f_{CS} = 169$ Hz. The negative sign arises from referencing diamagnetic D₂O to paramagnetic n-octanol. Figure 3 plots shift differences of the acetone line of the four test aliquots containing starting GdCl₃ concentrations from 0 to 10 mM. Doping shifts the D₂O acetone resonance increasingly downfield. From the linear least-squares fit shown in Figure 3, we find that $\Delta f = \Delta f_{CS}$ at [GdCl₃] = 2.56 mM.

A compensated layered biphasic system was prepared at this concentration. A 275 μ L volume of the denser D₂O component was injected into an NMR tube to fill it to the center of the detection region. Care was taken to avoid leaving water droplets on the tube wall. An equal volume of the octanol phase was then layered on top. The spectrum of this entire compensated layered sample, shimmed as described in the following section, is shown in Fig. 2B.

2.3. Bulk Shimming Biphasic Systems

A sucrose standard was used for initial shimming, and the biphasic sample was inserted afterward.⁵ Throughout shimming of the biphasic system, the acetone peaks in the two phases were kept separated approximately by Δf_{CS} . It is tempting to shim by forcing the acetone peaks to be coincident [16], but that is erroneous. Such a procedure actually warps the field, since it is clear that in a homogeneous field the lines will be separated by their solvent shift difference.

⁴By contrast, for an immiscible organic phase, only doping of the aqueous phase would be possible, so vortexing and separation would be unnecessary.

⁵It was realized in hindsight that an ideal shimming sample would be a portion of the matched D₂O component, extracted during preparation, and filled in a separate NMR tube to the same level as the biphasic system.

Satisfactory results were obtained by adjusting only Z_1 , Z_2 , and Z_3 . The effect of these shims on the two acetone lines in the biphasic sample parallels conventional shimming behavior with a homogeneous sample. There, odd and even Z-shims remove distortions that are symmetric or asymmetric, respectively about the center of the peak being shimmed. For the biphasic case, the shim symmetry center is the mid-frequency between the line pairs. Thus, odd Z-shims remove distortions common to both lines that trail in opposite frequency directions; even order shims remove distortions of the two lines that trail in the same frequency direction. Here, comparison between the shapes of the lines together provides guidance, rather than any particular feature of either line alone.

The procedure outlined above is suitable for coarse adjustment to optimize gross line shape features. For final adjustment at later stages, we simultaneously shimmed the sharp acetone peak in the octanol, and the D_2O lock level from the aqueous phase. This approach sufficed for achieving an acetone linewidth in the biphasic sample of 2.0 and 4.5 Hz for the n-octanol or D_2O phases, respectively, shown in figure 3. This is essentially the paramagnetically-broadened limit: using Topshim 3D to shim a tube filled only with aqueous phase yielded an acetone linewidth of 2.9 Hz. Although results shown in Fig. 3B do not have quite the same baseline quality as the capillary spectrum of Fig. 3A, line quality is adequate, and sufficient to resolve the ^{13}C sidebands.

2.4. Slice Selection Sequence

Unlike methods that rely on frequency-selective excitation [14–16], slice-resolved spectroscopy NMR was performed here using a 2D z-imaging pulse sequence illustrated in Figure 5. The 2D method we present incorporates the z-imaging portion of the gradient-encoding spin-warp sequence for spatial resolution [40]. Consequently, the 2D slice signal-to-noise (SN) ratio is the same as the SN ratio of an equivalent set of frequency-selected slices, but with every slice individually acquired for the time of the entire experiment. Also, the slice spectra are imaged without conflating spatial and chemical shift coordinates, so only conventional software is needed for post-processing [14, 16, 41].

On the other hand, all slices are imaged, whether of interest or not, which may require more time, and, for samples with strong lines, radiation damping occurring in the weak-gradient acquisition sets may introduce artifacts.

3. Results

1H 2D contour data (Fig. 6) were obtained on a 600 MHz Avance III spectrometer, using a Bruker Z855801 TXI probe and the stepped-gradient sequence of Fig. 5. In the F2 dimension, parameters were set to display spectra between 6 and 0 ppm. In the “F1” or z-dimension, the gradients were applied for 1.0 ms, in 64 steps over the control range $\pm 4.22\%$, which corresponded to a gradient range of ± 2.37 G/cm. With these parameters, 2D Fourier transformation yields spectra from 0.5 mm thick slices over a 32 mm overall window. Horizontal lines indicate slices, and their intersection with the peaks in contour view represents the spectra. The meniscus is located at the $z = 0$ mark.

Figure 6A shows the spectral contours for an uncompensated octanol/ D_2O system, bulk-shimmed as described above. Far from the interface, the attempt to force the acetone lines in the two phases to coalesce is marginally successful, in that the ridges in the outer regions do coincide at 2.35 ppm. Coincidence can be achieved over these relatively large regions because they are far enough apart to allow the shims to align the fields essentially independently. However, near the interface, distortion is unavoidable, since here the resonance frequencies jump suddenly in going from one phase to the other. The overall result is a gradient “jog” in the field near the interface, roughly the same as the undoped

shift difference measured in Fig. 3. The probe profile peak intensity is likewise distorted. The profile is a projection of maximum peak height, and this feature is affected by shim quality. Here we find that the profile from the stronger octanol phase appears weaker than the projection from the D₂O layer.

Figure 6B shows the same system, compensated with GdCl₃. The acetone lines at 2.37 and 2.47 ppm in the two phases have been shimmed to be separated by Δf_{CS} . There is virtually no visible line shape distortion at the interface. Further-more, the probe profile shown at the right of the figure accurately shows the strong ¹H signal in the octanol phase, terminating cleanly at the interface, where the phase changes to the ¹H-depleted deuterated aqueous phase.

4. Conclusion

We have presented a general method for paramagnetic doping of biphasic NMR systems to remove magnetic field distortion at the interface, suitable for any aqueous/organic system which can tolerate the presence of mM quantities of doping agent. The technique offers improved spectral resolution near the interface particularly for systems employing single-axis gradient coils for slice discrimination.

The procedure uses MAS to determine the susceptibility-matched target, and capillary tubes for calibration and achievement of match. The particular susceptibility calibration procedure we describe is tailored for systems where the phases are partially miscible, and where homogeneous solvent phases are desired. For the simpler case of immiscible phases, one is free to add any quantity of analyte at the outset, and only the aqueous phase need be doped, in isolation.

The use of acetone as a frequency marker for the purposes of this study was illustrative, and in fact, unnecessary. It should be clear in retrospect that any two lines f_O and f_A , taken from each phase, can be used in the same way we used the two different frequencies of the acetone resonance. The shift differences for any selected pair of resonances can be measured by MAS and capillary experiments, and compensated by doping, using the same procedure described above. Finally, we presented a simple 2D slice-spectroscopy sequence as an alternative to 1D frequency-selective excitation. The method offers increased SN per unit time, and clean separation of spatial and spectral information using conventional signal processing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- To address technical issues intrinsic to slice-resolved NMR spectroscopy of interfacial systems.
- Improved spectral resolution of biphasic samples near the interface.
- Shimming common analyte(s) in a heterogeneous system.
- To dispel reports in the literature that interfacial spectral distortion is due solely to surface chemistry.
- Introduce the use of paramagnetic salts to modulate bulk solvent properties.

Figure 1.

Simulation of the field transition near the interface between layers of a biphasic system. The phases are layered in an upright NMR tube placed coaxially in a homogeneous vertical magnetic field H_0 . Here z represents height from the idealized planar interface, and ρ the distance from the tube axis; lengths are normalized to the tube radius. The field experienced by nuclei in the sample shifts by $\mu_0 \Delta \chi H_0$ as one crosses from one phase to the other. The transition imposes a complex combination of axial and radial gradients. It is assumed that $\chi \ll 1$, so that only the z component of the B field parallel to H_0 is plotted.

Figure 2. Scheme of the strategy used to observe and match BMS across a biphasic sample. A. The chemical shift difference was isolated by MAS NMR. (Organic phase (O) in green and Aqueous phase (A) in blue.) B. From coaxial capillary shift measurements, a relationship between GdCl_3 concentration and BMS was determined. C. The GdCl_3 compensated biphasic sample was examined to confirm the susceptibility match.

Figure 3.

A. 1D spectrum of a sample of undoped D_2O stock solution fitted with a capillary tube insert containing D_2O -saturated octanol. B. Layered biphasic sample in a 5 mm NMR tube with a susceptibility matched aqueous layer and a D_2O -saturated octanol layer. Starting values of $[GdCl_3]$ are zero in A and 2.56 mM in B. The acetone lines in the aqueous phases are indicated with (*) and the acetone lines in octanol are referenced to 2.37 ppm. Doping shifts the aqueous acetone line downfield, from 2.20 ppm in the capillary system (A), to 2.47 ppm in the layered system (B) achieving $\Delta f = \Delta f_{CS} = +67$ Hz.

Figure 4. Gadolinium (III) chloride shift calibration. The shift difference for the acetone signal in an aqueous stock solution is plotted for starting $[\text{GdCl}_3]$ ranging from 0 to 10 mM. Dotted lines indicate the 2.56 mM doping level required to give an acetone shift difference Δf_{CS} .

Figure 5.

Basic stepped gradient pulse sequence. Timing is set to form an echo peaking at the start of the acquisition time. Resulting free induction decays (FIDs) are additionally phase modulated by a sequence of 1 ms gradient pulses ranging between ± 2.37 G/cm. Two-dimensional Fourier transformation of the resulting FID array yields a set of spectra from a stack of slices, each 0.5 mm thick.

Figure 6.

A 2D chemical shift image plot comparing data obtained without doping (A.) and with 2.56 mM GdCl₃ (B.). The acetone resonances, with lock active, follow the same behavior as in Fig. 3, where the aqueous acetone frequency shifts from 2.35 ppm in the undoped sample to 2.37 ppm and 2.47 ppm (organic and aqueous phases, respectively) with the addition of GdCl₃. Note the line shape improvement from the undoped to the doped system.